ELSEVIER

Etiologic and Early Marker Studies in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial

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ABSTRACT: The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, which is randomizing 74,000 screening arm participants (37,000 men, 37,000 women; ages 55–74) and an equal number of nonscreened controls, is a unique setting for the investigation of the etiology of cancer and other diseases and for the evaluation of potential molecular markers of early disease. At entry, baseline information is collected by questionnaire on dietary intake, tobacco and alcohol use, reproductive history (for women), family history of cancer, use of selected drugs, and other selected risk factors. Blood samples collected at the baseline screening exam are aliquoted to serum, plasma, red blood cell, and buffy coat fractions. At the next two annual screening visits, serum samples are collected. At the third annual reexamination, cryopreserved whole blood is obtained, in addition to serum, plasma, red blood cell, and buffy coat fractions. At the fourth

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and fifth years, serum, plasma, and buffy coat are collected. All blood samples are shipped to a central repository for long-term storage at −70°C. Dietary questionnaires and buccal cells for DNA analysis are obtained from nonscreened controls. Cancer cases are identified through annual follow-up questionnaires, and all deaths are identified through vital status tracing mechanisms. Procedures are being developed to obtain archival pathologic material for selected cases of cancer and related diseases. Initial investigations are focusing on the etiology of colorectal cancer and on the operative characteristics of tests for the early detection of colorectal and prostate cancer. *Control Clin Trials* 2000;21:349S–355S

KEY WORDS: *Etiology, genetic markers, health questionnaire, risk factors, dietary questionnaire*

BACKGROUND

Prospective epidemiologic studies are essential for increasing our understanding about the causes of cancer in humans [1–3]. Because questionnaire data and biologic samples are collected before the onset of clinical disease, the biases associated with retrospective research are eliminated. Also, because prospective research is not restricted to one or a few disease entities, risk factors can be studied in relation to the spectrum of associated diseases. This can be important when, for example, a particular genetic trait is associated with increased risk for one disease, but with decreased risk for another. A prospective epidemiologic investigation within the context of a cancer screening trial has the additional strength of relating the temporal development of disease (i.e., transition from screen negative to screen positive) to risk factor profiles, which is important because some risk factors may be more strongly associated with aggressive disease. As progress is made in understanding the causes of cancer in humans, it is becoming increasingly evident that the interplay of multiple genetic and environmental (in the broad sense of noninherited) factors are involved. To identify the population subgroups with combinations of genetic and environmental traits that lead to exceptionally high or low rates of disease, it is necessary to study relatively large numbers of participants, as are included in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, sponsored by the National Cancer Institute (NCI). The PLCO also provides opportunities to evaluate methods for the early detection of cancer and possibly to identify the determinants of nonmalignant diseases.

As the PLCO progresses, etiologic and early marker studies will be carried out to address hypotheses concerning potential carcinogenic and anticarcinogenic exposures and genetic susceptibility to disease risk. Questionnaire-based risk factor evaluations can be carried out for all trial participants. Biochemical and genetic studies of cancer etiology will typically involve comparison of risk factors between cases and a similar number of comparison subjects (nested case-control and case-cohort designs). Studies to evaluate the natural history of disease and to characterize early markers will be carried out utilizing the sequential collection of samples to relate biochemical changes in blood to the prediagnostic course of disease development.

METHODS

The etiology and early marker component is fully integrated with the early detection component of the PLCO. The etiologic and early marker component

is explained to PLCO participants, and they are offered the opportunity to participate in these additional studies of cancer and other diseases that affect their age group. Participation in the additional studies is, however, completely voluntary and has no impact upon their ability to take part in the screening component of the trial.

Baseline Questionnaires

A general risk factor questionnaire is administered to all study participants at trial entry to elicit information on demographics, body build, history of selected medical conditions and treatments, cancer screening history, family history of cancer, tobacco use, and occupation. Diet-related data are collected in a separate questionnaire, including information on usual consumption of foods, cooking practices, and use of nutritional supplements. To reduce costs, the questionnaires are self-administered and directly machine-readable. The individual screening centers (SCs) are responsible for administering the questionnaire to screened participants, reviewing questionnaires for completeness, and sending them or the machine-read results to a central processing facility for editing and merging with other trial data. An additional dietary questionnaire was added for screening arm participants in their fourth year on the trial and for control arm participants at trial entry. As the trial proceeds, additional questionnaires will be administered to screening and control arm participants to further elaborate on or to characterize changes in risk factor profiles.

Biologic Sample Collection

In addition to the blood collected for evaluation of prostate-specific antigen (PSA) in men and CA125 in women as screening tools, additional blood is collected from screening arm participants for etiologic and early marker studies. At the baseline (T0) screening exam, blood is collected and aliquoted into serum, plasma, red blood cell, and white blood cell (buffy coat) fractions. At the next two subsequent annual screening exams (T1 and T2), samples of serum are collected. At the T3 screen, cryopreserved whole blood is obtained, in addition to serum, plasma, red blood cell, and buffy coat fractions. All blood samples (except the whole blood sample for cryopreservation) are shipped once per month on dry ice to the PLCO biorepository at Frederick, Maryland for long-term storage at −70°C. The blood samples for cryopreservation are shipped on the day of collection by overnight mail to the biorepository.

Quality Control

The overall management of the PLCO trial, described elsewhere in this supplement [4, 5], provides a structure to maintain the quality of data and materials collected within the trial. Materials collection for etiologic and early marker studies are monitored at the SCs and at the coordinating center (CC). Questionnaires are usually reviewed for completeness. The general risk factor questionnaire includes a number of critical items that must be completed for each individual. During the processing of data, at the SCs and centrally, a series of checks are completed to assure highly standardized data quality. Each

Table 1 Status[a] of Materials Collection for Etiologic Studies in the Screening Arm of the PLCO

Participants randomized to screening	55,609
Participants screened	47,928
Questionnaires completed	>53,000
Baseline (T0) blood draw	45,556
T1 blood draw[b]	35,769
T2 blood draw[b]	24,443

[a] as of January, 1999.

[b] T1, T2, = first and second annual screening re-examinations, respectively.

aliquot of blood is tracked through a computerized system that links sample collection, sample shipment, and sample storage. Progress in data and sample collection is reviewed on a monthly basis.

TRIAL PROGRESS

Of those screened, about 95% have provided blood samples at the initial screening exam in addition to baseline dietary and general risk factor questionnaires (Table 1). Blood collections are also accruing from participants as they proceed through the subsequent screening rounds. Table 2 provides a projection of the number of selected cancers expected by 2010 in the screened and non-screened (control) arms.

As a long-term prospective trial, the PLCO has opportunities to modify and expand the materials collection for etiologic and early marker studies. Pilot and feasibility investigations have been carried out to evaluate the use of food frequency questionnaires, the collection at home of buccal cell samples from control arm participants, and the collection of viable lymphocytes from screened arm participants. Pilot investigations were necessary because trialwide implementation of protocol changes is very complex. In addition to increased staff and materials requirements, alterations are needed for each protocol change to the data collection forms and associated computerized data management system, which links SC and biorepository activities. The pilot investigators

Table 2 Expected Cases of Selected Cancers in the PLCO by 2010

	Men		Women	
Cancer site	Screened	Control	Screened	Control
All cancers	10,000	10,000	6,000	6,000
Prostate	2,000	2,000	—	—
Lung	2,000	2,000	650	650
Colorectal	1,000	1,000	650	650
Ovary	—	—	250	250
Breast	—	—	1,800	1,800
Bladder	750	750	150	150
Non-Hodgkin's Lymphoma	400	400	200	200
Stomach	250	250	100	100
Esophagus	120	120	30	30

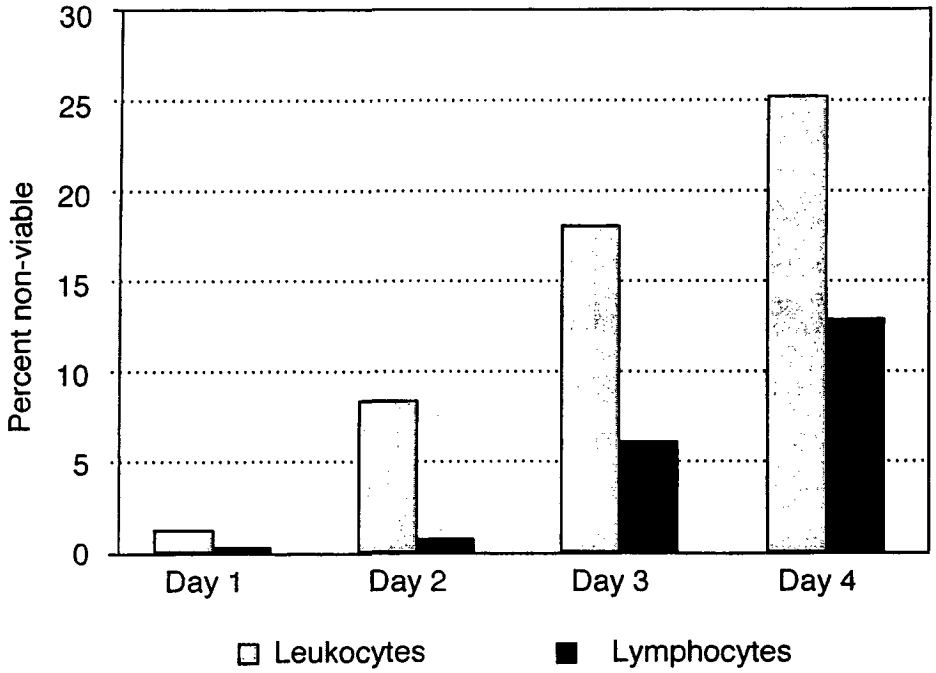

Figure 1 Pilot study: nonviable cells, by days to freeze.

addressed issues of technical feasibility, cost, and implementation of quality control systems.

A dietary questionnaire was administered at T0 only to the screened group. A pilot was initiated to evaluate response rates for an expanded dietary questionnaire administered to the screened group (their second dietary questionnaire) and, for the first time, to the control group. At each of three SCs, 150 screened and 150 control participants were selected for the pilot. Response rates for completion of the new dietary questionnaire were 84% in the screened group (range per SC: 68–95%) and 82% in the controls (range per SC: 72–89%). This questionnaire is now administered to the full screened arm and control arm populations.

The control group is as large as the screened group, but these participants do not contribute blood samples because they do not come to the SCs for exams. A pilot collection of buccal cells from control participants at home as a DNA resource was a modification of a previously developed protocol [6]. Three hundred controls (100 at each of three SCs) were mailed informed consent documents, a buccal cell collection kit, and instructions for collection and shipment. The collection kit consisted of a 1.5-ounce bottle of a commercial mouthwash and a 15-mL collection cup. Participants were instructed to pour about 8 mL of the mouthwash into the collection cup (to a premarked fill line), rinse, and then spit the sample back into the cup. Informed consents were returned to the SCs, while the buccal cell samples were sent to a central repository. When samples were received at the repository, volumes were recorded and DNA was extracted. In the pilot, 87% of the informed consent documents and

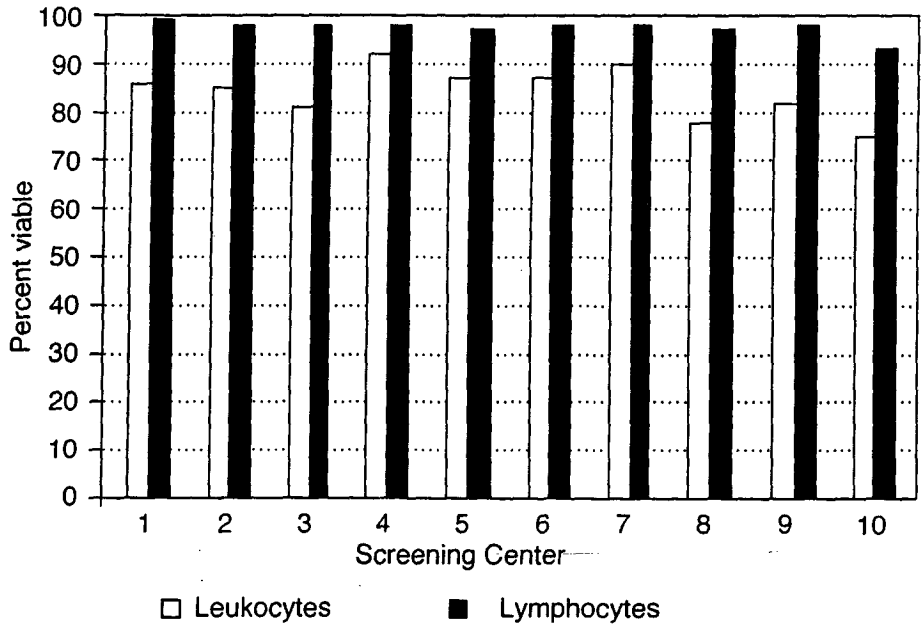

Figure 2 Viability by screening center.

87% of the collection kits were received. Median DNA yields were greater than 20 μg per sample, with only about 5% of samples providing less than 5 μg DNA. As proof of the quality of the received sample DNA, polymerase chain reaction (PCR) amplification for common polymorphisms vitamin D receptor, alcohol dehydrogenase 3 (VDR, ADH_3) were attempted, with 87% and 95% success rates, respectively. Buccal cell collection from all participants in the control arm is underway.

DNA retrievable from a 10-mL blood sample is adequate for a substantial number of PCR-based assays. With developments in the characterization of the human genome, it may be expected, however, that large-scale DNA analyses will become feasible in population-based epidemiologic studies. We are developing methods for the collection of blood samples that, in principle, can provide a replenishable DNA supply. Whole blood (20 mL in acid-citrate-dextrose) is collected at the SCs and shipped by overnight delivery to an NCI-designated lab for processing, where they are mixed with dimethyl sulfoxide (DMSO) (0.1 mL per 1.0 mL of blood), aliquoted in 1.8-mL volumes into 2.0-mL NUNC cryovials, submitted to a controlled rate freeze, and stored in gas phase N_2. In developing this technique, we showed by propidium iodide staining and flow cytometry that lymphocyte viability was excellent for at least 2 days for blood held at room temperature (Figure 1). We found that shipment from the SCs usually took place within 2 days (98% of samples shipped) and that lymphocyte viability was maintained during shipment (Figure 2). Epstein-Barr Virus (EBV) transformation was tested on 20 samples received from the SCs after storage for at least 1 month, with a 100% success rate. Longitudinal studies continue to assess long-term storage effects on lymphocyte viability and responsiveness to EBV transformation.

PLANNED RESEARCH

The NCI is supporting etiologic investigations of the major cancers that occur in this population. Etiologic investigations are underway regarding factors associated with the colorectal adenoma-carcinoma sequence. Investigations of prostate, lung, and breast cancer etiology will follow as cases of these cancers accrue in the cohort. Investigation of PSA variants has been concluded. As potential markers for the early detection of cancer are developed to the point where testing in this prospective trial is appropriate, special investigations will be carried out to determine assay sensitivity and specificity and to describe other operative characteristics of the marker. The population data will also be of use for the evaluation of hypotheses about the determinants of diseases other than cancer.

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